glutamine synthesis, and (iv) lower beta-hydroxybutyrate level in cerebral cortex which has been subjected to cerebral ischemia.

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5 ~~24~~. (New) The transgenic mouse of claim 4 ~~23~~, wherein the promoter is platelet derived growth factor (PDGF)-B-chain promoter.

6 ~~25~~. (New) The transgenic mouse of claim 4 ~~23~~, wherein the DNA sequence which encodes amyloid-beta peptide alcohol dehydrogenase is a human DNA sequence.

REMARKS

Claims 1, 2, 5, 6, 10 and 14-16 are pending. In this Amendment, applicants have cancelled claims 1, 2, 5, 6, 10 and 14-16 and added new claims 17, and 19-25 corresponding thereto, respectively and new claim 18. New claims 17-25 have been added to introduce certain format changes. Likewise, applicants have amended the specification to introduce certain formatting changes. Upon entry of this amendment claims 17-25 will be pending and under examination. Applicants detail below the amendments made herein.

The Examiner previously alleged in a December 20, 2001 Office Action that the specification cites multiple references to RAGE transgenic mice. The Examiner stated that appropriate correction is required.

In response, applicants amended the specification to correct the obvious errors in a June 20, 2002 Amendment. After further reviewing the specification, applicants have found additional references to RAGE and applicants have hereinabove amended the specification to correct an obvious error at page 4, line 21;

page 4, line 25; page 6, lines 5, 28 and 29; page 7, lines 1, 9 and 10; and page 49, line 12 of the specification. The specification now recites "ABAD" rather than "RAGE." In support of such an amendment to the specification, applicants respectfully direct the Examiner to the Summary of the Invention, and in particular page 3, lines 3-10 which recites as follows:

> "The present invention provides for a transgenic non-human animal whose cells contain a recombinant DNA sequence comprising a nerve tissue specific promoter operatively linked to a DNA sequence which encodes *amyloid-beta peptide alcohol dehydrogenase (ABAD)*, wherein said non-human exhibits at least one phenotype from the group consisting of: overexpression of ABAD, elevated levels of basal ATP; protection from metabolic or ischemic stress" (emphasis added).

Accordingly, applicants contend that the Summary of the Invention at page 3, lines 3-10, as a summary of the presently claimed invention, demonstrates that the recitation of "RAGE" was an obvious error and such amendment to the specification does not introduce any new matter.

Page 17, line 21 of the specification recites the term "inschemic" and applicants have hereinabove amended the specification to recite "ischemic" which is the correct spelling of the term.

Applicants note that the specification contains two sets of figure legends (pages 4-7 and pages 77-82), of which only the first corresponds to the figures. Consequently, applicants have amended the specification hereinabove to delete both the set of

figure legends found on pages 77-82 and references to said figure legends in the text of the specification (pages 61-71), i.e., at page 63, line 29; page 64, lines 2, 4, 25 and 26; page 65, lines 4, and 12-15; and page 72, line 12.

Applicants also note that the specification has two lists of references (pages 72-77 and pages 84-89).

To more clearly point out that the references recited on page 72-77 of the specification are related to Example 4 of the Detailed Description and that the references recited on pages 84-89 of the specification are related to Example 1 of the Detailed Description, applicants have amended the specification hereinabove. Page 72, line 31 now recites "References for Example 4" and page 84, line 1 now recites "References for Example 1". Applicants maintain that such an amendment to the specification does not introduce any new matter.

Applicants have canceled claims 1, 2, 5, 6, 10, and 14-16 without prejudice and have added new claims 17-25. Support for new claim 17 can be found *inter alia* on page 3, lines 3-10; and page 45, line 13 through page 46, line 5. Support for new claim 18 can be found *inter alia* on page 45, line 13 through page 46, line 5. Support for new claim 19 can be found *inter alia* on page 8, lines 28-30. Support for new claim 20 can be found *inter alia* on page 3, lines 3-10; page 8, lines 11-24 and lines 32 and 33; page 9, lines 10-14; page 17, lines 9-15; page 44, lines 8-23; page 44, line 31 through page 45, line 11; page 51, line 31 through page 52, line 6; page 69, lines 25-27; page 70, lines 2-6; and page 73, lines 8-20. Support for new claim 21 can be found *inter alia* on page 8, lines 28-30. Support for new claim 22 can be found *inter alia* on page 3, lines 3-10; page 8, lines 11-24 and lines 28-33; page 9, lines 10-14; page 17,

lines 9-15; page 44, lines 8-23; page 44, line 31 through page 45, line 11; page 51, line 31 through page 52, line 6; page 69, lines 25-27; page 70, lines 2-6; and page 73, lines 8-20. Support for new claim 23 can be found *inter alia* on page 9, lines 3-14. Support for new claim 24 can be found *inter alia* on page 8, lines 28-30. Support for new claim 25 can be found *inter alia* on page 9, lines 26-28. Applicants maintain that these amendments raise no issue of new matter.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the enclosed amount of $430.00, is deemed necessary in connection with the filing of the accompanying RCE. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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EXHIBIT A - MARKED-UP VERSION OF THE SPECIFICATION

Paragraphs beginning on page 4, line 14 and ending on page 4, line 28:

-- **Figures 4A, 4B, 4C and 4D.** ABAD expression in Tg PD-ABAD mice (+) compared with nontransgenic littermate controls (-). Figure 4A (Northern) and Figure 4B (Western) analysis of homogenates of cerebral cortex. Equal amounts of RNA (note approximately equal intensity of 28S ribosomal RNA band on the ethidium bromide stained gel) and protein were loaded in each lane. Figures 4C-4D show immunohistochemical identification of [RAGE] <u>ABAD</u> in cerebral cortex from a Tg PD-[RAGE] <u>ABAD</u> mouse (Figure 4C) and a nontransgenic littermate control (Figure 4D).

Figure 5. [RAGE] <u>ABAD</u> expression in brain subregions of Tg PD-ABAD mice compared with nontransgenic littermate controls (nonTg). Immunoblotting was performed protein extracts of brain homogenates derived from the indicated brain subregion. --

Paragraph beginning on page 5, line 25 and ending on page 6, line 6:

-- **Figures 7A-7D.** Induction of stroke in Tg PD-ABAD mice. Figures 7A-7B. Tg PD-ABAD mice and nonTg littermates were subjected to middle cerebral artery occlusion and were evaluated 24 hrs after the ischemic insult to determine neurologic deficit score (Figure 7B), and, following sacrifice, infarct volume (Figure 7A). Figures 7C-7D. At the same time point, cerebral cortex was harvested to determine ATP, lactate and ß-hydroxybutyrate (BHB) levels determined on extracts of whole brains (from animals subjected to the stroke procedure 24 hrs previously) from Tg

PD-ABAD or nonTg control mice (N=5, in each case). Data is reported as the mean ± SD ($P<0.04$ for ATP and $P<0.03$ for lactate). Transient middle cerebral artery occlusion model of stroke in mice: comparison of infarct volume in Tg PD-[RAGE] ABAD and nontransgenic littermate controls (nonTg). *$P<0.05$. --

Paragraphs beginning on page 6, line 27 and ending on page 7, line 11:

-- **Figures 11A-11B.** Semiquantitative analysis of synaptophysin immunoreactivity in hippocampus of Tg PD-[RAGE] ABAD/hAPP, Tg PD-[RAGE] ABAD, Tg hAPP, and nontransgenic littermate control mice at 4 months of age.

Figures 12A1, 12A2, 12A3, 12A4, and 12B. Increased expression of activated caspase-3 in cerebral cortex from Tg PD-[RAGE] ABAD/hAPP mice. Figures 12A1-12A4, immunostaining for activated caspase-3. Figure 12B, quantitation of immunocytochemical results from multiple fields of all mice in each of the experimental groups. Scale bar, 10 µm.

Figures 13A-13B. Northern analysis (Figure 13A) and immunoblotting (Figure 13B) of E16 cortical neuron cultures with ^{32}P-labelled human ABAD cDNA (Figure 13A) or anti-human [RAGE] ABAD IgG (Figure 13B). (+) indicates neurons obtained from Tg PD-[RAGE] ABAD mice and (-) indicates neurons are from nontransgenic littermate controls. --

Paragraphs beginning on page 17, line 17:

-- The following procedure is carried out: Introduction of ABAD into neurons or other cells (we did this also in cultured COS cells as described hereinbelow in the

Examples), increases their resistance to metabolic stress as represented by an [inschemic] ischemic microenvironment, excitotoxic stress or nutritional stress (decreased glucose). We have recently obtained data that shows in a kainate model of brain injury, neuronal loss is less in these ABAD transgenic mice.

Paragraph beginning on page 49, line 10:

-- In each case, the *in vitro* and *in vivo* systems based on Tg PD-ABAD mice or cells derived from them are ideal for studying [RAGE] ABAD inhibitors, as well as for dissecting contributions of ABAD to physiologic/pathophysiologic outcomes. --

Paragraphs beginning on page 63, line 20 and ending on page 65, line 20:

-- ABAD metabolism of ß-hydroxybutyrate. The broad enzymatic properties of ABAD as an oxidoreductase suggested that it might facilitate cellular utilization of ketone bodies, such as D-ß-hydroxybutyrate, a major energetic substrate during nutritional deprivation *in vivo*. First, we tested DL-ß-hydroxybutyryl-CoA, a known substrate of bovine liver-derived hydroxyacyl-CoA type II (HADH II)/ABAD (17), with our purified *E. Coli*-derived human recombinant ABAD[; the reaction fit Michaelis-Menton kinetics with K_m was _134 _M and V_{max} _26.3 _mol/min/mg (Fig. **1A**)]. The latter result, obtained with a racemic DL mixture of ß-hydroxybutyryl CoA, was similar to that observed previously with the bovine liver HADH II (17). Using the same preparation of recombinant ABAD, we then studied D-ß-hydroxybutyrate[; the reaction also fit best to Michaelis-Menton kinetics with K_m was _3.7 mM, and V_{max} was _4 nmol/min/mg (Fig. **1B**). With the L-form of ß-hydroxybutyrate, K_m was -_1.6 mM, and V_{max} _3.5 nmol/min/mg (Fig. **1C**)]. In a purified system, ABAD is clearly more effective with ß-hydroxybutyryl-CoA as a

substrate (presumably this is the L-form, which is an intermediate in the fatty acid ß-oxidation pathway in mitochondria). However, depending on physiological conditions, certain substrates may turn out to be more abundant, such as D-ß-hydroxybutyrate during periods of starvation when levels of ketone bodies are elevated and, thus, could become relevant. In fact, plasma levels of ß-hydroxybutyrate are reported to reach the millimolar range in animals and humans subject to nutritional deprivation (21-23). Furthermore, ß-hydroxybutyryl CoA generated by acyl CoA dehydrogenase is another likely substrate of ABAD in a cellular milieu rich in ß-hydroxybutyrate. Thus, ABAD would appear to have the potential to be pivotal for enhancing metabolism of ß-hydroxybutyrate.

<u>Characterization of COS cells stably-transfected to overexpress ABAD</u>. COS cells provided a useful model to test our concept that ABAD modulated the cellular response to nutritional stress because of their low endogenous expression of ABAD; low levels of mRNA were present [(Fig. **2A**, lane 4)] and no antigen was detectable [(Fig. **2B**, lane 7)] in lysates of wild-type COS cells. Following stable transfection with either pcDNA3 alone (vector), pcDNA3/wtABAD (encoding wild-type ABAD) or pcDNA3/mutABAD (encoding a mutant form of ABAD devoid of enzymatic activity; 14), cells were plated at limiting dilution and clones were prepared. Three types of clones were established, those expressing vector alone (COS/vector), wild-type ABAD (COS/wtABAD) and mutant ABAD (COS/mutABAD). Studies were performed with three representative clones of each type of stably-transfected COS cell. Whereas COS/vector cells displayed low levels of ABAD transcripts [(Fig. 2A, lanes 3)] and antigen [(Fig. 2B, lanes 6)], comparable to control COS cells, COS/wtABAD cells showed high levels of ABAD mRNA [(Fig. 2A, lanes 2)] and antigen [(Fig. 2B, lanes 3-5)]. Subcellular fractionation studies on COS/wtABAD cells [(Fig. **2C**, line *a*)] demonstrated the presence of ABAD both in fractions 1-2 enriched for the endoplasmic reticulum marker GRP78 [(Fig. 2C, line *c*)] and in the mitochondrial pellet (fraction 6) containing cytochrome c [(Fig. 2C, line

e)]. Similar experiments performed with COS/mutABAD stable transfectants [(Fig. 2C, line *b*)] displayed high levels of ABAD transcripts [(Fig. 2A, lane 1)] and antigen [(Fig. 2B, lanes 1-2)] in a distribution analogous to that seen in COS/wtABAD cells. These data indicated that in COS/ABAD stable transfectants, the enzyme is present at the same sites previously observed in cells endogenously expressing ABAD or those transiently transfected to overexpress ABAD (13,14,16,19).

Paragraph beginning on page 72, line 10 and ending on page 73, line 6:

Increased expression of ABAD in human brain following cerebral infarction and in response to experimentally-induced cerebral ischemia [(Fig. 5)] suggests that induction of ABAD might subserve normal protective mechanisms. In view of the complexities of cellular metabolic pathways, it was necessary to prove that ABAD could promote metabolic homeostasis in response to nutritional deprivation. ABAD-transfected COS cells displayed increased energy charge and flux of acetyl-CoA through the TCA cycle in medium containing ß-hydroxybutyrate compared with controls in which the active site of ABAD was mutationally inactivated. Enhanced metabolic homeostasis was reflected by maintenance of MTT reduction and morphologic phenotype in ABAD-transfected COS cells. Similarly, transgenic mice overexpressing ABAD in cortical neurons demonstrated increased flux of acetyl-CoA through the TCA cycle following ß-hydroxybutyrate infusion compared with nontransgenic littermates. However, increased basal levels of ATP (and energy charge; data not shown) in brains of Tg PD-ABAD mice, even before nutritional stress, was unexpected, and suggests a more general protective potential of ABAD in response to a range of environmental challenges. This apparent increase in the overall energy charge in the presence of ABAD, implies that the enzyme may render neurons metabolically more stable and, thus, less susceptible to fluctuations in substrate availability. --

Page 74, line 31:

-- REFERENCES <u>FOR EXAMPLE 4</u> --

Page 86, line 1:

-- References <u>for Example 1</u> --